REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claim 1 has been amended in this paper. Therefore, claims 1-15 are pending. Of these claims, claims 12-15 are withdrawn as being directed at a non-elected invention. Accordingly, claims 1-11 are under active consideration.

Claims 1-6, 9 and 10 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) and Berlin et al (WO01/27317, published 4/1/2001)." In support of the rejection, the Patent Office states the following:

> Citations from Berlin et al (WO01/27317, published 4/1/2001) refer to the National Stage (U.S. Patent No. 7,179,594 issued 2/20/2007). The National Stage is deemed an English language translation of the PCT.
>
> Genomic methylation pattern is interpreted to include-tissue specific methylation patterns.
>
> The amendment to the claims to recite hemimethylation requires the presence of a methylated and non-methylated strands.
>
> The amendment of "said DNA being methylated at one or more cytosine positions" is not defined in the specification. Thus "said DNA being methylated at one or more cytosine positions" is being given the broadest reasonable interpretation of a single or double stranded DNA with at least one cytosine that is methylated.
>
> Lopez et al teaches the amplification of genomic DNA by PCR in the presence of a DNA methyltransferase (see figure 1 and page 17, lines 26-28) (claim 1) and amplification by single strand displacement amplification and methylation with a DNA methyltransferase (see page 18, line 10-16) for detection. PCR and single strand displacement amplification are interpreted as steps B-C of claim 1. The strands synthesized by chain extension or single

strand displacement contain the methylated parent strand and synthesized strand, which is not methylated and thus are hemimethylated. Lopez teaches ^{3}H-s-adenosyl methionine as a methyl donor with a detectable label (see page 4, line 2) (claim 4 and 5). Lopez et al further teaches the use of anchored PCR primers on a solid matrix to create ordered maps (see page 21 lines 2-4) (claim 6). Lopez et al teaches the treatment of amplified targets with methylation sensitive restriction enzyme capable of distinguishing methylated and non-methylated cytosines (see page 32, lines 25-29).

Lopez et al does not teach the use of DNA methyltransferase that preserves methylation status of genomic DNA, providing a sample DNA with one or more methylated cytosines (claim 1). Lopez does not specifically teach analyzing the methylation status to determine the methylation status of the starting sample (claim 1, step g).

Lopez et al does not teach the use of DNMT1 a maintenance methyltransferase (claims 2 and 3). However, Pradhan et al teaches the use of DNMT1 as a methyltransferase (see abstract). Pradhan teaches maintenance methylation "ensures propagation of tissue specific methylation patterns during development" (see page 33002, first column text, lines 8-10). Pradhan teaches that DNMT1 has a higher reaction velocity for hemimethylated DNA substrates (see page 3302, 2nd column, last paragraph). Pradhan thus teaches DNMT1 is a maintenance methyltransferase ensures propagation of specific methylation patterns. Pradhan further teaches cytosine methylation is important in embryonic development, carcinogenesis and genetic disease (see page 33002, 1st column of text lines 1-5). Pradhan thus teaches maintenance methylation and the methyltransferases that maintain methylation patterns are important in embryonic development, carcinogenesis and genetic disease. Pradhan teaches the use of DNA known to be methylated (page 33006, 2nd column, last paragraph).

Berlin teaches methods of distinguishing methylation changes at the 5 position of cytosine bases (column 1, lines 9-10). Berlin teaches that cytosine methylation regulates transcription, genomic imprinting and tumorigenesis (column 1, lines 33-35). Berlin teaches detection of methylation by methylation sensitive restriction digestion (column 1, lines 53-67). Berlin teaches methylation detection by bisulfite treatment (column 2, lines 22-51). Berlin teaches the use of genomic DNA. Berlin further teaches 5

methylcytosine is the most common genetic modification in eukaryotic cells (1st column, lines 33-34).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the DNMT1 methyltransferase taught by Pradhan as the methyltransferase in Lopez's method of amplification and methylation of eukaryotic genomic DNA because Pradhan teaches DNMT1 is a maintenance methyltransferase that ensures propagation of methylation patterns. It would have been further prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the amplified DNA with the genomic methylation pattern generated by the combination of Pradhan and Lopez in the methods of detecting methylation taught by Berlin. The ordinary artisan would be motivated to use the DNMT1 of Pradhan with Lopez method of Pradhan's method of methylating and amplifying DNA with the genomic methylation pattern. The artisan would be motivated to analyze the amplified DNA with the genomic methylation produced by the method of Lopez and Pradhan in the method of Berlin, because it would allow for detection of methylation patterns and thus further understanding of genomic imprinting, transcriptional regulation and tumorigenesis as taught by Berlin and Pradhan. The artisan would have a reasonable expectation of success as they are merely replacing one methyltransferase for another in methods of amplifying and methylating DNA and using known methods of detecting methylation.

Applicant respectfully traverses the subject rejection.

Claim 1, from which claims 2-6, 9 and 10 depend, has been amended in this paper and now recites "[a] A method for cytosine methylation analysis of genomic DNA wherein the genomic template DNA is amplified such that the cytosine methylation pattern of the genomic template DNA is retained in the amplificate sequence(s), said method comprising the following steps:

(a) providing a sample of DNA, said DNA being methylated at one or more cytosine positions;

(b) heating the genomic DNA to a temperature operative to cause denaturation;

(c) cooling the denatured DNA in the presence of single stranded oligonucleotide primers such that the primers anneal to the DNA;

(d) heating the mixture in the presence of a polymerase and nucleotides to a temperature such that the primers are extended, thereby resulting in hemimethylated DNA;

(e) contacting the hemimethylated DNA with a methyltransferase and a methyl donor molecule under conditions conducive to the methylation of the synthesised strand such that the CpG dinucleotides within the synthesised strand are methylated according to the methylation status of the corresponding CpG dinucleotide on the template strand thereby preserving the genomic methylation pattern;

(f) repeating steps (b)-(e) a plurality of times to reach a plurality of nucleic acids, whereby each of said nucleic acids is methylated at the same one or more cytosine positions as the DNA provided in step (a); and

(g) analyzing the methylation of the nucleic acids of step (f) whereby the methylation of the DNA of the sample of step (a) is deduced."

Support for the present amendment to claim 1 may be found in the present specification, for example, on page 7, lines 11-15; on page 6, lines 21-23, and on page 21, lines 29-30.

As best understood by Applicant, the Patent Office appears to be asserting that it would have been obvious to replace the standard methyltransferase enzyme in a method suitable to detect mutations (disclosed by Lopez), which is totally unsuitable for the purpose of a template's methylation detection at all, with DNMT1 (disclosed by Pradhan as an enzyme acting as maintenance methyltransferase *in vivo*) to result in the claimed method.

However, Applicant respectfully submits that this line of reasoning is clearly based on hindsight. There is no motivation at all to even start with the method of Lopez et al., as Applicant will demonstrate below.

The Patent Office argues that the motivation to replace the transferase of Lopez with the maintenance transferase would be given by the teaching of Pradhan that maintenance methylation "ensures propagation of tissue specific methylation patterns during development." However, even assuming arguendo, that this alone would be motivation to exchange a methyltransferase (a point Applicant does not concede), it would only make the method of the present invention obvious if the method to start from would have been suited to address the same problem, i.e., analysis of "native" genomic methylation status (not of a later produced methylation status). This is not the case, as will be shown below.

To find out whether something is obvious, one should focus on the problem to be solved, on the aim in mind, the need in the art. That is why it is of high importance to first identify the closest state of the art (defined as having the same or very similar intention, being from the same field, and ideally having a number of features in common). If you start from there and see whether the modification the inventor made to this state of the art in order to solve the problem has been suggested in the art and could have been performed without major effort, you may consider it as obvious.

It is the aim of the claimed method to enable the analysis of methylation patterns of (limited amounts of) genomic DNA as found *in vivo* which require amplification prior to being analysed, which has now been reflected in the amended version of claim 1:

> A method for cytosine methylation analysis ~~the amplification~~ of genomic DNA wherein the genomic template DNA is

amplified such that ~~whereby~~ the cytosine methylation pattern of the genomic template DNA is retained in the amplificate sequence(s), said method comprising the following steps:

The problem solved by the present method is to allow the analysis of *in vivo* methylation patterns in amounts of template DNA that are so small that they require a pre-amplification prior to analysis. The subject of the invention, therefore, is to provide an amplification method which conserves/maintains the methylation pattern of genomic template DNA (instead of diluting it with each round of amplification).

The need for amplification of small amounts of DNA is routinely solved by PCR amplification. However, in a standard PCR, the inherent methylation information carried by the genomic template is lost because, when copying the single strands, the polymerase ignores the subtle difference between a methylated and an unmethylated cytosine. After several cycles of PCR, there are so many copies of unmethylated strands produced that the now hemi-methylated template/copy duplex is diluted to such an extent that it is not reliably detectable anymore. However, the reliable detection of the in vivo methylation pattern of genomic DNA is the intent of the method of the present invention. Dilution of the genomic DNA carrying the methylation pattern of interest has the opposite effect of what the aim of the invention is.

In the prior art, the problem of "lost" (i.e., tremendously diluted) methylation information in PCR reactions has been solved by the use of a bisulfite pre-treatment. The bisulfite treatment modifies the unmethylated cytosines in the genomic template differently than it modifies the methylated cytosines. As a result, the polymerase treats them differently when copying the strands. The closest prior art, therefore, is the method described by Berlin at al., which describes the bisulfite pretreatment as the method of choice to allow amplification of genomic DNA while

maintaining the methylation information.

The problem in the art was to identify an alternative to the well-established and reliable two-step procedure of bisulfite pre-treatment followed by PCR amplification, in order to amplify the template DNA without losing or diluting the methylation information and to follow this up with a methylation analysis.

There is no teaching in the art that allows multiplying the template sequence, without losing its methylation status, unless you translate the cytosine methyl-modification into a modification which is not lost during amplification with a polymerase.

In order to solve the problem, a person of ordinary skill in the art might, therefore, have tried to convert the methylated cytosines or(!) the unmethylated cytosines (selectively) into some other nucleotide (or find a similar way to mark them differentially).

In any case, to address this problem, a person of ordinary skill in the art would not (!) consider using the method of Lopez et al. as a starting point, as it clearly does not care for the *in vivo* methylation pattern of the genomic DNA, which is diluted in the PCR to an extent that it could not be reliably analyzed anymore. Instead, the method of Lopez creates a new, *in vitro* methylation pattern as a tool to identify mutations of methylation sites. This is a completely different intention. The fact that in some prior art methylation analysis is compared to mutation analysis is solely based on the pre-treatment with bisulfite, which translates the methylation differences in sequence differences. But as this is the step which is replaced in the method of the invention, this cannot count as an argument for obviousness.

The method of Lopez is about "painting" specific restriction sites (see page 1, lines 10-18) by the use of methyltransferases. They only get "painted" when they are not mutated. The intent of

the method clearly is "genotyping."

In the outstanding Office Action, page 8, lines 9 and 10, the Patent Office states that "[t]he teachings of amplification by Lopez are relied upon in the instant method, and not the genotyping." However, Applicant respectfully submits that this does not make sense, as the Patent Office continues to stress that a person of ordinary skill in that art only had to exchange the methyltransferase of the method of Lopez with the methyltransferase disclosed by Pradhan. The use of methyltransferases in Lopez et al., however, very clearly belongs to the genotyping and not the amplification part. For example, in each and every example disclosed in the application, the amplification is performed prior to the treatment with the methyltransferase, in other words, prior to the marking of those sequence variations, i.e., the "painting of restriction sites," i.e., the genotyping.

The method of Lopez is based on a standard PCR amplification or strand displacement, wherein all (but a minute amount) of the methylation information is lost (or diluted to an extent that it cannot be reliably detected anymore) after that point, as discussed above. Therefore, it is not suited to solve the problem and, indeed, belongs to a different field of art.

On page 7 of the outstanding Office Action, the Patent Office states that, when starting with a methylated DNA (as required by the claim),

> some of the amplified DNA would be hemimethylated as the methylated starting DNA would still be present and thus would provide a substrate for the hemimethylation sensitive restriction site for the DNMT1 of Pradhan.

Only the -after several cycles- heavily diluted original template molecules still bear some of the methylation information (as hemimethylated remainders). But, these very few molecules do not play any role in the method of Lopez et al. Neither their presence nor their fate is discussed any further in the art. Instead, these must be neglected as they only present a tiny amount of side

product.

Therefore, when discussing obviousness, the mere presence of these few remaining molecules cannot be a reason to choose Lopez as closest prior art, as a starting point to develop a methylation analysis method.

Lopez merely suggests -as a possible variation of his method- that adding a heat-stable methyltransferase during the PCR amplification could bear advantages. However, there is no evidence or data provided, showing that this, indeed, works in a reliable way. The application contains a number of examples, but none of these examples disclose said suggested variation of the method; hence, it cannot be considered a proper teaching that a PCR would even work in the presence of a methyltransferase.

In addition, the methyltransferase of Pradhan is labile and thus subject to degradation at moderate temperatures (see page 33005, 1st column, 1st full paragraph). Therefore, if a person of ordinary skill in the art intended to detect methylation, he would not use a method based on PCR amplification of the template as disclosed in Lopez et al.

Without knowledge, i.e., without hindsight of the solution to the problem, the ordinarily skilled scientist would not have taken Lopez et al. as a basis to search for a methylation retaining PCR, as his method is targeting a completely different problem, such as sequence variation analysis, genotyping. On page 8, lines 11-14, of the outstanding Office Action, the Patent Office responds to Applicant's statement that Lopez is non-analogous art by stating the following:

> Berlin's method clearly indicates that distinguishing methylation and cytosine to thymine mutations are related (see column 1, lines 9-13).

Applicant respectfully submits that the foregoing statement from Berlin is clearly taken out of

context. In fact, the passage in question merely says:

> The present invention concerns a method for distinguishing 5-position methylation changes of cytosine bases and cytosine-to-thymine mutations and for detecting single nucleotide polymorphisms (SNPS) or point mutations in genomic DNA.

Berlin clearly refers to DNA which has been pre-treated with bisulfite in order to translate methylation changes in sequence variations. Without such a pre-treatment, however, the DNA variations of mutation and methylation are very different, especially in the sense that mutations can be copied by polymerase activities whereas methylation differences cannot. In fact, Berlin points out that only after that conversion all the techniques based on base pairing behavior, such as PCR, can be employed for analyzing methylation:

> A relatively new method which has been used most frequently in the meantime for investigating DNA for 5-methylcytosine is based on the specific reaction of bisulfite with cytosine, which is converted into uracil after subsequent alkaline hydrolysis, and uracil corresponds to thymidine in its base pairing behavior. 5-Methylcytosine, in contrast, is not modified under these conditions. Thus, the original DNA is converted in such a way that methylcytosine, which cannot originally be distinguished from cytosine by its hybridization behavior, now can be detected by "standard" molecular biological techniques as the single remaining cytosine, for example, by amplification and hybridization or sequencing. All of these techniques are based on base pairing, which can now be fully exploited.

Therefore, Berlin also teaches away from using a method based on base pairing behavior, such as PCR amplification, for the analysis of methylation without performing a suitable pretreatment beforehand.

This is why any art which requires a PCR amplification of the template DNA as a first step must be regarded as not qualifying as a good starting point to develop a method suitable for

methylation analysis, unless you pre-treat the DNA in a manner allowing for the differentiation for example with bisulfite.

Therefore, the closest state of the art is an amplification method comprising the step of pre-treatment with bisulfite. Such a method has the same aim, i.e., analyzing the methylation patterns of genomic DNA as it occurs in vivo, and also the same feature of solving the problem of limited amounts of template to analyze by amplification with a polymerase. Starting from here, the difference from the method of the present invention is to be determined as follows: The difference to the closest prior art is a) to not pre-treat the genomic DNA, and b) instead, to perform the steps A-D of the first cycle of a PCR reaction and to add a methylation maintenance methyltransferase and to repeat this cycle a number of times until sufficient amounts have been amplified.

Applicant does not see how this solution would have been obvious. This is, in part, because Applicant has proceeded contrary to accepted wisdom by replacing a widely accepted well working pre-treatment step. None of the references teach the advantageous and unexpected result that a polymerase mediated chain reaction would work in presence of a heat labile methylation maintaining methyltransferase. There is no suggestion in the prior art to apply a maintenance methyltransferase to hemi-methylated DNA *in vitro*. The fact that Pradhan teaches the role of the enzyme in nature as being important in embryonic development, carcinogenesis and genetic cause is, in itself, not a teaching or a suggestion to use the enzyme as a technical tool in a method of molecular biology targeting the amplification of small amounts of template DNA. Proceeding contrary to accepted wisdom of the prior art, as Applicant has done, is strong evidence of unobviousness. In re Hedges, 228 U.S.P.Q. 685 (Fed.Cir. 1986), citing *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 220 U.S.P.Q. 303 (Fed.Cir. 1983), citing *United States v Adams*, 148 U.S.P.Q. 479

(1966).

Accordingly, the evidence of record strongly rebuts the *prima facie* case for obviousness as asserted in the Office Action. At least in view of the foregoing reasons, it is clear that the combination of Lopez et al. and Berlin et al., even in view of Pradhan et al., teaches away from the method of the present invention.

Pradhan et al. is limited to disclosing the discovery of an enzyme with methylation maintenance capabilities and disclosing its role in biology. Pradhan teaches that cytosine methylation is important in embryonic development, carcinogenesis, and genetic disease, i.e., in biological processes occurring in a living cell. Pradhan simply describes the role of this enzyme in nature, which is not necessarily what an enzyme does *in vitro*. Pradhan is completely silent about the possibility to use this enzyme as a molecular biology tool in order to analyze cytosine methylation patterns. There is no suggestion in the art as to any technical use of this enzyme.

Pradhan, therefore, does not say that the enzyme is heat resistant or stable in conditions of PCR, nor does Pradhan suggest using it as a tool in molecular biology at all.

In fact, instead, Pradhan teaches away from using the DNMT1 methyltransferse in a PCR reaction, as Pradhan describes it as labile and subject to degradation at moderate temperatures (see page 33005, 1st column, 1st full paragraph). A PCR, however, requires several cycles of denaturing at temperatures which are not moderate at all.

Therefore, even knowing about the existence of DNMT1 and its role in nature as a methylation maintenance enzyme (from Pradhan et al.), when trying to modify the closest prior art, i.e., methods such as disclosed in Berlin et al., with the aim of amplifying an amount of template DNA, a person of ordinary skill in the art would not have added this methyltransferase enzyme to a

PCR reaction, which requires harsh denaturing temperatures which are likely to inactivate an enzyme other than a polymerase anyway, without inventive skill.

Therefore, there is no motivation given to select said enzyme and replace the well working bisulfite pre-treatment of the method(s) disclosed by Berlin et al. with a new and potentially unreliable enzymatic treatment, which would have to be carried out simultaneously with the PCR and would also carry the potential to interfere with the PCR reaction itself, as it has not been taught in the art that a PCR would work in presence of a maintenance methyltransferase, nor any methyltransferase. Lopez merely suggests -as a possible variation of its method of detecting sequence variations- that adding a heat stable methyltransferase during the PCR amplification could bear advantages (with regards to speed and simplicity).

As it has nowhere been suggested to modify the PCR amplification reaction itself, in order to maintain the methylation pattern of the genomic template DNA, neither by Lopez nor by Pradhan, the method which is subject of the invention must be regarded nonobvious.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 7 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) and Berlin et al (WO01/27317, published 4/1/2001) as applied to claim 1-6, 9, and 10 above, and further in view of Shatkin et al (US Patent 6312926)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez, Pradhan and Berlin are set forth above. Lopez, Pradhan and Berlin do not teach the methyltransferase immobilized on a solid support.

> However, Shatkin et al teaches the use of hMET (methyl transferase) immobilized on protein G beads for washing assays (see column 24, lines 3-12).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez, Pradhan and Berlin method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized methyltransferase taught by Shatkin, because Shatkin teaches immobilization allows washing of assays. The ordinary artisan would be motivated to improve Lopez, Pradhan and Berlin method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized methyltransferase or polymerases as taught by Shatkin, because Shatkin teaches immobilization allows washing of assay and detection of protein interactions.

Applicant respectfully traverses the subject rejection. Claim 7 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al., Pradhan et al. and Berlin et al. for at least the reasons given above. Shatkin et al. fails to cure all of the deficiencies of Lopez et al., Pradhan et al. and Berlin et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 7 is patentable over the applied combination of Lopez et al., Pradhan et al., Berlin et al. and Shatkin et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 8 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) and Berlin et al (WO01/27317, published 4/1/2001) as applied to claims 1-6, 9, and 10 above, and further in view of Stemple et al (WO/2000/53805)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez, Pradhan and Berlin are set forth above. Lopez, Pradhan and Berlin do not teach the polymerase immobilized on a solid support.

> However, Stemple teaches the immobilization of a polymerase on a solid support (see page 3 lines 14-15). Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously (See page 7, lines 25-26).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez, Pradhan and Berlin method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilizing a polymerase as taught by Stemple, because Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously. The ordinary artisan would be motivated to improve Lopez, Pradhan and Berlin method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized polymerases as taught by Stemple, because Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously.

Applicant respectfully traverses the subject rejection. Claim 8 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al., Pradhan et al. and Berlin et al. for at least the reasons given above. Stemple et al. fails to cure all of the deficiencies of Lopez et al., Pradhan et al. and Berlin et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 8 is patentable over the applied combination of Lopez et al., Pradhan et al., Berlin et al. and Stemple et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) and Berlin et al (WO01/27317, published 4/1/2001) as applied to claims 1-6, 9, and 10 above, and further in view of Gonzalgo et al (US Patent 6251594)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez, Pradhan and Berlin are set forth above. Lopez, Pradhan and Berlin do not teach the use of bisulphate solution to distinguish methylation status of cytosine bases.
>
> However, Gonzalgo et al teach the use of bisulphite to distinguish methylated and unmethylated cytosines (column 7, lines 5-6). Gonzalgo teaches the use of bisulphite is quantitative, does not use restriction enzymes, and allows multiplexing (see column 7, lines 7-10).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez, Pradhan and Berlin method of amplifying genomic DNA while maintaining and distinguishing genomic methylation patterns by use bisulphite solutions taught by Gonzalgo, because Gonzalgo teaches the use of bisulphate is quantitative, does not use restriction enzymes, and allows multiplexing. The ordinary artisan would be motivated to improve Lopez, Pradhan and Berlin method because, the use of bisulphite is quantitative, does not use restriction enzymes, and allows multiplexing. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the instant invention was made, it must be considered that said ordinary skilled artisan would have had reasonable expectation of success in practicing the claimed invention.

Applicant respectfully traverses the subject rejection. Claim 11 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al., Pradhan et al. and Berlin et al. for at least the reasons given above. Gonzalgo et al. fails to cure all of the deficiencies of Lopez et al., Pradhan et al. and Berlin et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the applied combination of Lopez et al., Pradhan et al., Berlin et al. and Gonzalgo et al.

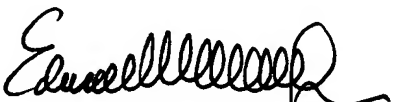
Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: 

Edward M. Kriegsman
Reg. No. 33,529
30 Turnpike Road, Suite 9
Southborough, MA 01772
(508) 481-3500

Dated: September 21, 2009

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on September 21, 2009.

Edward M. Kriegsman
Reg. No. 33,529
Dated: September 21, 2009